A retrotransposon sequence is related to DNA instability in barley microspore culture

María Muñoz[1], Luís Cistué[1], Ana-María Castillo[1], Ignacio Romagosa[2] & María-Pilar Vallés[1*]

[1]Departamento de Genética y Producción Vegetal. Estación Experimental de Aula Dei, C.S.I.C., Apartado 202, E-50059 Zaragoza, Spain. [*]Author to whom correspondence should be addressed (e-mail valles@eead.csic.es).
[2]Area de Conreus Extensius, Centre UdL-IRTA, E-25198 Lleida, Spain

Abstract
In order to assay gametoclonal variation in barley, a set of double haploid (DH) plants (cv. Igri) regenerated from isolated microspore culture by direct embryogenesis, was especially developed. AFLP analysis was performed with eleven enzyme-primer combination, that represented the study of 719 band polymorphisms. Only one change in the band patterns was observed, indicating a high degree of genetic stability. An extra band was present in DH lines regenerated from microspores, treated or not with sodium azide, representing a "hot spot" of instability. The sequence of this band was related to barley "sukkula" retrotransposon element. Our preliminary results suggest the activation of retrotransposon by barley isolated microspore culture.

Key words:
Barley - Doubled haploid - Genetic stability - Microspore culture - Retrotransposon

Introduction

Doubled haploid (DH) production is a value tool for plant breeders because it provides a rapid way to produce a large number of homozygous plants at any stage in a breeding program, (Thomas et al., 2003). Furthermore, DHs have been very useful for mutation, gene mapping and genomic studies. Androgenesis, which involves the regeneration of plants through a tissue culture phase, is the most efficient method for barley DH lines production.

It is well documented that some tissue culture methods generate genetic modifications such as numerical and structure changes of chromosomes, gene mutation, activation of transposable elements and also changes in DNA methylation patterns (Lee & Phillips, 1988).

Although early studies described genetic modifications in barley plants obtained by androgenesis, this instability was attributed to a callus regeneration phase. However, in systems where plants were regenerated by direct embryogenesis, there was little evidence of induced genetic changes, suggesting that point mutations and larger DNA rearrangements had not occurred but differences in levels of methylation (Devaux et al., 1993; Logue, 1996)

In the present work, a molecular analysis of barley DH lines, produced from isolated microspore, was performed in order to broaden the few reports related to gametoclonal variation obtained by direct embryogenesis.

Materials and Methods

Plant material and isolated microspore culture

The model cultivar Igri was chosen for this study due to its good androgenic response. Dissected anthers were pre-treated with 0.7 M mannitol (Cistué et al. 1994). Microspore isolation, culture and plant regeneration was performed as described by Castillo et al. (2000). Treatment with 10^{-5} M sodium azide was applied for 1 h according to Castillo et al. (2001).

DNA extraction and AFLP analysis

Leaf-DNA was extracted using minor modifications of the CTAB procedure according to Saghai-Maroof et al. (1984). AFLP analysis was performed according to AFLP Analysis System I (Invitrogen) specifications. Eleven primer-enzyme combinations were used for AFLP analysis (E33M61, E35M47, E36M50, E36M59, E40M49, E40M50, E41M49, E35M60, E37M61, E41M60, E35M48). Acrylamide gels were silver stained. AFLP-band was cloned using the pGEM-TEasy vector system I (Promega) and sequenced.

Results and Discussion

Of the different methods used to detect DNA changes, AFLP analysis was chosen due to its high level of polymorphism. Other methods as RFLP and RAPD have been used before in the same cultivar Igri without success (Devaux et al 1993).

In order to rule out any other possible variability source, as pre-existing variation in the starting material or additional stress caused by mutagenic treatment, a complete set of phenotypically normal DH lines were developed. From a single Igri DH plant (I-DH), six daughter plants (I-DH1 to I-DH6) were grown. The plant I-DH2 was used as the donor plant for microspore isolation and self-pollinated seeds production (I-DH2a1 to I-DH2a5). DH plants were regenerated from microspore treated (I-DH2sat1 to I-DH2sat15) or not (I-DH2mc1 to I-DH2mc15) with sodium azide, after isolation. Igri plants developed from commercial seeds (Is) were also included in the analysis.

The study of eleven primer-enzyme combinations, that represented the analysis of 719 band polymorphisms, revealed only one change in the band patterns, with the presence of an extra band of 320pb in the E36M50 combination, indicating a high degree of genetic stability (Figure1).

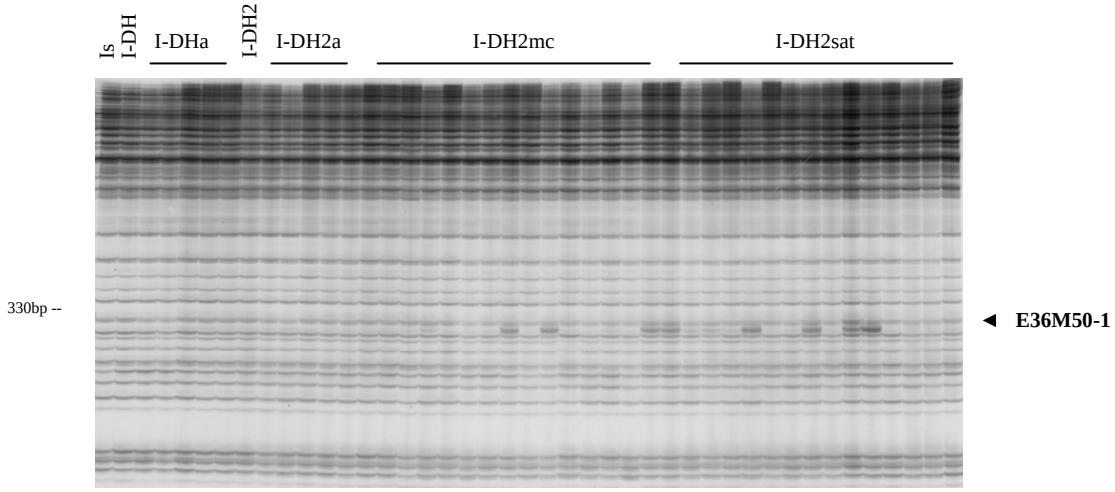

Figure 1: Identification of AFLP-band polymorphism in barley DH plants obtained by isolated microspore culture.

The amplified band was clearly present in three I-DH2mc and five I-DH2sat lines out of fifteen. The presence of the band in independent DH lines indicate that this band represent a hypervariable “hot spot” of DNA instability (Linacero et al., 2000). The higher number of lines with additional band in the sodium azide treated material, could indicate that the mechanism of this instability induction is related to stress pressure, increasing the rate of induction with severe stress conditions.

In order to know the sequence involved in the new AFLP polymorphism the band was excised, cloned and sequenced. The cloned band corresponded with a 294 bp fragment that

showed homology to different barley retrotransposon “sukkula” elements. The Sukkula family is unusual, having terminal sequences similar to LTR terminal regions of rice gypsy-like retrotransposons (RIRE), but lacking a protein-coding domain (Shirasu et al. 2000).

Substantial reports have shown that retroelements are activated under different stress and specifically during cell and tissue culture (Grandbastien 1998). However, as far as we know, there is only one report on transposon activation in DH lines derived from anther culture, where rice plants were regenerated through a callus phase (Kikuchi y col. 2003).

These preliminary results suggest the activation of retrotransposon by barley isolated microspore culture. Further analysis should be performed in order to clarify the mechanism generating this genetic variability.

Acknowledgements
We thank Dra. S. Prat from CNB-CSIC (Spain) for help with cloning and sequencing. María Muñoz was supported by a fellowship from FPI (FP-2001-1877) MEC of Spain. The research was supported by grants AGF 99-1024 and AGL2001-1631 from CICyT of Spain.

References

Castillo, A.M., M.P. Vallés & L. Cistué, 2000. Comparison of anther and isolated microspore cultures in barley: Effects of culture density and regeneration medium. Euphytica 113: 1-8.

Castillo, A.M., L. Cistué, M.P. Vallés, J.M. Sanz, I. Romagosa & J.L. Molina-Cano, 2001. Efficient production of androgenic doubled haploid mutants in barley by the application of sodium azide to anther and microspore cultures. Plant Cell Reports 20:105-111.

Cistué, L., A. Ramos, A.M. Castillo & I. Romagosa, 1994. Production of large number of doubled haploid plants from barley anthers pre-treated with high concentrations of mannitol. Plant Cell Reports 13: 709- 712.

Devaux, P., A. Kilian & A. Kleinhofs, 1993. Anther culture and Hordeum bulbosum-derived barley doubled haploids: mutations and methylation. Molecular General Genetics 241:674-679.

Grandbastien, M.A., 1998. Activation of plant retrotransposons under stress conditions. Trends Plant Science 3:181-187.

Kikuchi, K., K. Terauci, M. Wada & H-Y. Hirano, 2003. The plant MITE *mPing* is mobilized in anther culture. Nature 421:167-170.

Lee, M. & R.L. Phillips, 1988. The chromosomal basis of somaclonal variation. Annual Review Plant Physiology Plant Molecular Biology 39:413-437.

Linacero, R., E. Freitas Alves & A.M. Vázquez, 2000. Hot spots of DNA instability revealed through the study of somaclonal variation in rye. Theoretical. Applied Genetics 100:506-511.

Logue, S.J., 1996. Genetic stability in microspore-derived doubled haploids. In: S. Mohan Jain, S.K. Sopory & R.E. Veilleux (Ed.), In vitro Haploid Production in higher Plants, Vol 2: Applications, pp.1-51. Kluwer Academic Publishers, Dordrecht, Boston, London.

Shirasu, K., A.H. Schulman, T. Lahaye & P. Schulze-Lefert, 2000. A contiguos 66-Kb barley DNA sequence provides evidence for reversible genome expansion. Genome Research 10:908-915.

Thomas, W.T.B, B.P. Foster & B. Gertsson, 2003. Doubled haploids in breeding. In: M. Maluszynski, K.J. Kasha, B.P. Forster & I. Szarejko (Ed.), Doubled Haploid Production in Crop Plants, A Manual, pp. 337-349. Kluwer Academic Publishers, Dordrecht, Boston, London.